SCIENTIFIC LETTER

Clopidogrel reduces platelet–leucocyte aggregation, monocyte activation and RANTES secretion in type 2 diabetes mellitus

S A Harding, J Sarma, J N Din, P M Maciocia, D E Newby, K A A Fox

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Patients with diabetes mellitus have an increased risk of developing atherosclerosis and its sequelae. Atherosclerosis is an inflammatory disease involving multiple interactions between platelets, leucocytes and endothelial cells.[1] Clopidogrel, a specific antagonist of the ADP $P2Y_{12}$ receptor, inhibits both platelet activation and aggregation induced by ADP.[2] Although clopidogrel has well-documented antithrombotic actions, its potential anti-inflammatory effects have been little investigated. We examined whether specific platelet inhibition with clopidogrel would reduce systemic inflammatory markers and specifically platelet, monocyte and endothelial activation in patients with type 2 diabetes mellitus.

METHODS

We enrolled 20 patients with type 2 diabetes mellitus without clinical evidence of cardiovascular disease, malignancy, chronic inflammatory disorders, intercurrent illness, renal or hepatic insufficiency or contraindications to clopidogrel who had not taken antiplatelet agents within the preceding two weeks. Ethical approval was obtained from the local ethics committee and all participants provided written informed consent. They were treated with clopidogrel 75 mg daily for 28 days. Fasting peripheral venous blood samples were obtained at baseline and at 28 days.

Plasma concentrations of soluble CD40 ligand (sCD40L) (Bender MedSystems) and sE-selectin (R&D Systems) were determined by enzyme-linked immunosorbent assays. Plasma concentrations of the chemokine regulated on activation normal T cell expressed presumed secreted (RANTES, CCL5) were assessed with the human chemokine flow cytometric bead array (BD Biosciences).

To evaluate CD40L and P-selectin on platelets, whole blood was diluted 1:10 with phosphate-buffered saline (PBS) and incubated with anti-CD42a:fluorescein isothiocyanate (FITC) (Serotec), anti-P-selectin:phycoerythrin (PE) (DakoCytomation), anti-CD40L:PE (DakoCytomation) and appropriate isotype controls for 20 min before the cells were fixed with 1% paraformaldehyde. To evaluate CD40 and CD11b on monocytes, blood was diluted 1:2 with PBS and incubated with the following monoclonal antibodies: anti-CD14:FITC (Serotec), anti-CD40:PE (Serotec), anti-CD11b:PE (Serotec) and appropriate isotype-matched controls for 20 min. Thereafter, samples were fixed and the red cells lysed by the addition of FACS-Lyse (Becton Dickinson). To determine platelet–monocyte and platelet–neutrophil binding, whole blood was diluted 1:2 with PBS and incubated with anti-CD14:PE (DakoCytomation) and anti-CD42a:FITC or isotype-matched controls for 20 min at room temperature, before FACS-Lyse was added. Platelet–monocyte and platelet–neutrophil aggregates were defined as monocytes or neutrophils positive for CD42a. At least 2500 cells were measured by flow cytometry (EPICS XL2; Beckman-Coulter).

Samples were analysed with EXPO 32 software (Cytometry Systems).

Continuous variables are reported as mean (SD). Data were statistically analysed with a paired t test for normally distributed variables or the Wilcoxon matched pairs test for non-parametric variables. GraphPad Prism (GraphPad Software, San Diego, California, USA) was used for all statistical analyses. Significance was taken at $p < 0.05$.

RESULTS

Participants (51 (7) years) had a diagnosis of diabetes for 6 (4) years with mean haemoglobin A1c of 7.9 (1.4)% indicating moderate glycaemic control. Baseline and four-week fasting plasma glucose concentrations, haemoglobin A1c and fasting lipid profiles were similar (data not shown, NS).

Clopidogrel treatment reduced platelet surface P-selectin (5.6 (2.8)% v 3.7 (1.8)%, p = 0.002) but not CD40L expression (3.3 (0.7)% v 3.4 (1.0)%, p = 0.7). Monocyte surface expression of CD40 (40.7 (5.9)% v 35.0 (6.5)%, p = 0.007) and CD11b (74.8 (7.5) v 60.0 (5.9) mean fluorescence intensity, p = 0.02) were reduced after clopidogrel treatment. Clopidogrel treatment reduced both platelet–monocyte (23.2 (7.6)% v 17.8 (7.4)%, p = 0.01) and platelet–neutrophil (7.7 (2.9)% v 6.0 (2.3)%, p = 0.04) binding (fig 1).

Plasma concentrations of the platelet-derived chemokine RANTES (3722 (1123.0) pg/ml v 1476 (1229) pg/ml, p < 0.0001) were greatly reduced. However, concentrations of sCD40L (0.24 (0.42) ng/l v 0.23 (0.32) ng/l, p = 0.24) and sE-selectin (67.8 940.3) ng/ml v 70.0 (40.5) ng/ml, p = 0.44) were unchanged after clopidogrel treatment.

DISCUSSION

Over the past decade it has become apparent that complex signalling occurs between platelets, leucocytes and endothelial cells, and that these interactions have proinflammatory and proatherosclerotic consequences.[1][3] Xiao and Théroux[4] have previously reported that clopidogrel attenuated the excess platelet–monocyte and platelet–neutrophil conjugates in patients with acute coronary syndromes. Consistent with these findings, our results show that clopidogrel treatment in patients with diabetes mellitus also reduces platelet–leucocyte conjugates despite considerably lower levels of platelet activation. In addition, we have shown that clopidogrel was associated with less monocyte surface expression of CD40 and CD11b indicating reduced monocyte activation. Reduction of platelet–leucocyte interactions and monocyte

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Abbreviations: FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline; PE, phycoerythrin; RANTES, regulated on activation normal T cell expressed presumed secreted; sCD40L, soluble CD40 ligand
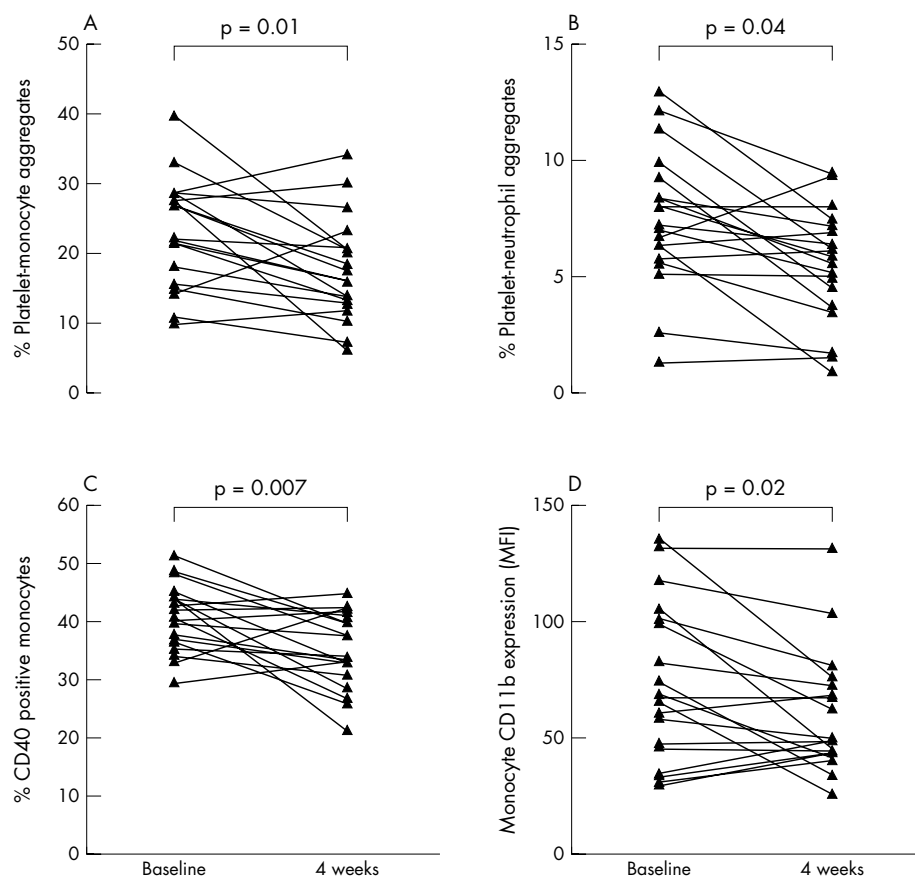
Figure 1 (A) Platelet–monocyte aggregates, (B) platelet–neutrophil aggregates, (C) monocyte surface expression of CD40 and (D) monocyte surface expression of CD11b before and after four weeks' treatment with clopidogrel in patients with type 2 diabetes. MFI, mean fluorescence intensity.

activation may well contribute to the clinical benefits of clopidogrel.

RANTES mediates monocyte and lymphocyte recruitment to sites of vascular injury and has a key role in the progression of atherosclerosis. RANTES may be secreted by several cell types including activated platelets and leucocytes.[3] In our study clopidogrel reduced both platelet and monocyte activation and it is therefore not surprising that clopidogrel also greatly reduced plasma RANTES concentrations. This reduced RANTES secretion provides another potential anti-inflammatory mechanism that may contribute to the clinical benefits of clopidogrel.

CD40L has been shown to mediate a broad range of proinflammatory and prothrombotic responses. In contrast to P-selectin and RANTES, neither platelet surface expression of CD40L nor sCD40L was reduced by clopidogrel. Variable effects of clopidogrel on sCD40L have previously been reported. Xiao and Théroux[4] reported a 27% reduction in plasma concentrations of sCD40L in patients with acute coronary syndromes, whereas Quinn et al[5] found that clopidogrel did not affect pre- or post-procedure sCD40L concentrations in patients undergoing percutaneous coronary intervention. In the present study, baseline platelet activation and sCD40L levels were substantially lower than in the acute coronary syndrome population studied by Xiao and Théroux,[4] in which the sCD40L reductions were greatest with baseline concentrations > 0.5 ng/ml. In our study only one participant had an sCD40L concentration > 0.5 ng/ml and in this patient clopidogrel caused a 23% reduction. These findings suggest that clopidogrel may not have the same effect on CD40L in patients with lower baseline levels of platelet activation and sCD40L.

In conclusion, clopidogrel treatment in patients with type 2 diabetes mellitus not only reduces platelet activation but also reduces platelet–leucocyte interactions, monocyte activation and plasma concentrations of the chemokine RANTES. These findings provide further support for the hypothesis that clopidogrel has important anti-inflammatory actions that may contribute to its clinical benefits.

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IMAGES IN CARDIOLOGY ....

Cardiac compression from a rheumatoid pericardial mass

A 75-year-old man presented with progressive dyspnoea. There was a 24-year history of severe deforming rheumatoid arthritis for which he had required a number of disease modifying therapies. At the time of admission his symptoms were controlled on non-steroidal anti-inflammatory drugs (NSAIDs) and prednisolone. He had no clinical signs of cardiac tamponade on presentation; however, a chest radiograph showed an enlarged cardiac silhouette and bilateral pleural effusions. A transthoracic echocardiogram and computed tomographic (CT) scan of the thorax (left panel) confirmed a large loculated pericardial effusion with compression of the right ventricular cavity. Aspiration of pleural fluid confirmed a transudate with a normal glucose. Pericardiocentesis resulted in an improvement in his symptoms. No malignant cells were seen and he was discharged for outpatient follow up. His symptoms recurred as did the pericardial effusion and he was transferred to a cardiothoracic centre for surgery. At operation a large and friable pericardial mass was debrided and a pericardial window was performed. Histology revealed fibrinous pericardium and chronic inflammatory infiltrates (middle and right panels). There was a further recurrence at four weeks and despite further drainage and aggressive immunosuppression he did not recover and the postmortem examination confirmed bilateral pulmonary emboli.

Pericardial compression in rheumatoid arthritis is rare. Pericardiocentesis and the formation of a pericardial window only provide temporary improvement and we suggest that the definitive treatment should be a pericardectomy. To our knowledge this is the only description of a histologically proven rheumatoid inflammatory mass presenting as cardiac compression.

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CT scan of the thorax showing a large pericardial collection compressing the right ventricle. There are also bilateral pleural effusions.
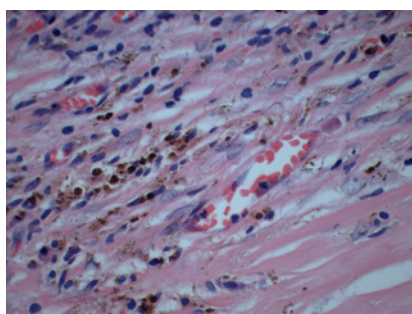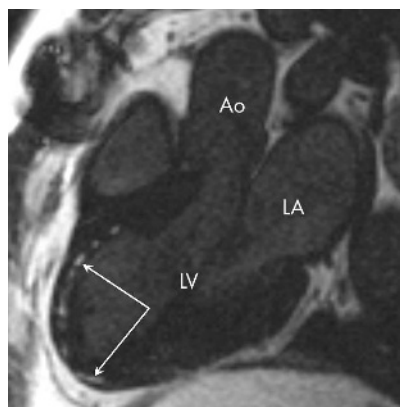
Haematoxylin and eosin stain showing fibrous pericardium containing large numbers of haemosiderin laden macrophages and a patchy chronic inflammatory infiltrate.
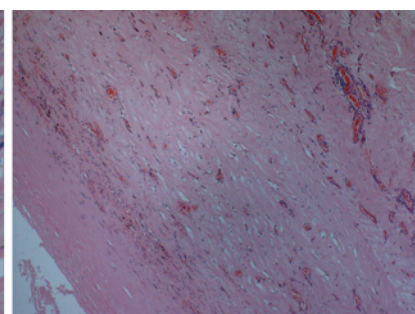
Lipomatous metaplasia in myocardial infarction detected by cardiovascular magnetic resonance

A 64-year-old man with a prior history of anterior wall myocardial infarction treated with thrombolysis in 1992, followed 10 years later by coronary artery bypass surgery, underwent cardiovascular magnetic resonance examination (CMR) for quantification of residual left ventricular function. Steady state free precession cine-CMR demonstrated thinned myocardium with dyskinesis of the distal anteroseptal and apical left ventricular regions consistent with an old myocardial infarction. Areas of unusual signal intensity compared to that of the surrounding myocardium were noted on cine-CMR within the infarcted region. These areas were confirmed to be composed of adipose tissue due to their characteristic high signal intensity on T1-weighted turbo spin echo images and complete disappearance of signal using fat suppression. The adipose tissue also had high signal intensity on two-dimensional inversion recovery images obtained before administration of gadolinium-based contrast (see panel) and was surrounded by areas of myocardial fibrosis demonstrated on late contrast-enhanced (LCE) images. These findings are consistent with lipomatous metaplasia in the region of an old myocardial infarction. This case demonstrates that a high signal intensity on LCE images may not always represent fibrosis alone in regions of old myocardial infarction.

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Left ventricular outflow tract view; two-dimensional inversion recovery image before administration of contrast, arrows indicating high signal intensity in infarcted area. Ao, aorta; LA, left atrium; LV, left ventricle.